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Review Article

Utility of salivary biomarker for stress induced by dental treatment

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Summary To appropriately and smoothly perform dental treatment, the objective evaluation of patients' stress during treatment is important, for which effective means and methods to measure stress-related markers in simply and noninvasively collectable saliva have been investigated. In this review, saliva was collected from pediatric patients before and after treatment, and sIgA, α-amylase, and cortisol were measured to evaluate the stress of dental treatment on them. On analysis of the duration and content of treatment in dental records, the sIgA level showed a clearer response to stress compared to α-amylase and cortisol. Measurement of changes in the salivary sIgA level may be a useful, noninvasive stress evaluation method, and sIgA may serve as a stress marker.

Contents

Introduction

The sound and pain of cutting in dental treatment increase patients' fear and anxiety. This becomes trauma, and leads to avoiding visiting a dentist due to feelings of stress, even if an oral disease is present. Particularly, stress may induce maladjustment behavior in children and interfere with dental treatment. Reduction of the stress of dental treatment may promote the adaptability of patients and enable them to comfortably receive dental treatment, subsequently contributing to the maintenance of a favorable oral condition [1—11].

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Studies on stress have been performed in many fields, and methods to evaluate stress using stress-related substances contained in blood, urine, and saliva as indices have been attracting attention. When the body is loaded with stress, 2 stress reaction systems are activated: the sympathetic nervous—adrenomedullary system (SAM system) and hypothalamic—adrenocortical system (HPA system) [12]. When a stress is loaded, the SAM system releases catecholamines, such as noradrenaline, and the HPA system releases cortisol. IgA is an index of the immune system and strongly influenced by the SAM and HPA systems [1,13—16]. IgA can be analyzed in saliva, and its relationships with cigarette smoking, the frequency of alcohol intake, and biological stress, such as bacteria and infectious diseases, have been reported [17,18].

Saliva has recently been attracting attention as a sample for stress-related substance measurement because its collection is less invasive, safer, and easier than blood sampling. Stress-related substances contained in saliva include chromogranin A (CgA), secretory immunoglobulin A (sIgA), and salivary amylase, in addition to catecholamine and cortisol [19—27]. Among the stress-related materials present in saliva, sIgA has been identified [26]. The regulation of sIgA secretion and synthesis is not only dependent on prior antigenic stimulation, but also under strong neuroendocrine control [27]. Therefore, the mechanism of the salivary sIgA response to stress is comparatively simple.

In this review, salivary sIgA measurement is mainly described as a method to accurately evaluate dental treatment-induced stress in patients. The establishment of an objective method to evaluate the stress of dental treatment facilitates the elucidation of causes of fear and anxiety for dental treatment and their prevention, and it is necessary for children to comfortably receive dental treatment.

Evaluation of stress of dental treatment in children

The subjects were 4—11-year-old children (19 boys and 10 girls) who visited the pediatric outpatient dental clinic of Osaka Dental University Hospital. The objective of the study was sufficiently explained, and consent for saliva sampling was obtained from the children and their parents.

This study was conducted in accordance with the guidelines of the Osaka Dental University Ethics Committee (No. 050353). Saliva samples were collected using sterilized rolled-cotton, Salivette.

Salivary sIgA was measured using an enzyme-linked immunosorbent assay (ELISA) kit, which was designed to produce an immune complex of an immobilized antibody against the secretory component and an enzyme-labeled antibody against IgA. The absorbance of the chromogenic product at 492 nm, corresponding to the amount of sIgA, was measured using a spectrophotometer. The sIgA level was determined as the concentration of salivary sIgA divided by the concentration of total protein [28].

Salivary cortisol was assayed using Salivary Cortisol ELISA Assay Kit (SALIMETRICS LLC, PA, USA). Salivary α-amylase activity was assayed using Salivary α-Amylase ELISA Kit (SALIMETRICS LLC, PA, USA). Each value was corrected at the concentration of total protein.

The medical records of the dental treatments were checked in detail regarding the following information: (1) sex, (2) age, (3) treatment time, (4) use of infiltration anesthesia (used or not used), (5) type of operation (surgical or non-surgical), (6) degree of pain (painful or painless), and (7) behavior (cooperative or uncooperative).

The changes in sIgA levels after treatment were compared to assess their utility for evaluating the stress caused by dental treatment. Differences in changes in sIgA levels were evaluated statistically using the Wilcoxon signed-ranks test, whereas differences between two groups within each categorized subject were evaluated employing the Mann-Whitney *U*-test.

The changes in salivary sIgA, cortisol, and α-amylase levels after treatment in the 29 patients are shown in Fig. 1. Overall, 83% of the subjects showed a decrease in their salivary sIgA levels following the dental treatment. Furthermore, the levels were significantly decreased after the treatment compared to the corresponding levels before treatment [29].

Dental treatment-induced changes in the sIgA and cortisol levels were more marked than that in the α-amylase level [30].

In dental treatment, the control of stress in patients is important for avoiding some secondary disadvantages, such as a loss of motivation for dental treatment. In previous studies, attempts to analyze some biological markers during dental treatment were performed in children. It was shown that salivary noradrenaline increased significantly when the children sat in a dental chair and subsequently received infiltration anesthesia [1], and that salivary cortisol levels at various stages of dental treatment were significantly higher compared with a control group not receiving any dental treatment [31]. Although stress assessment by questionnaires and physiological indexes has been attempted, there are no useful methods for evaluating the latent stress suffered by patients. Although part of the sIgA, as well as cortisol, shifts from the blood to the saliva, the majority of the sIgA is directly synthesized in and secreted by the salivary glands [32]. Therefore, it is likely that sIgA can react rapidly

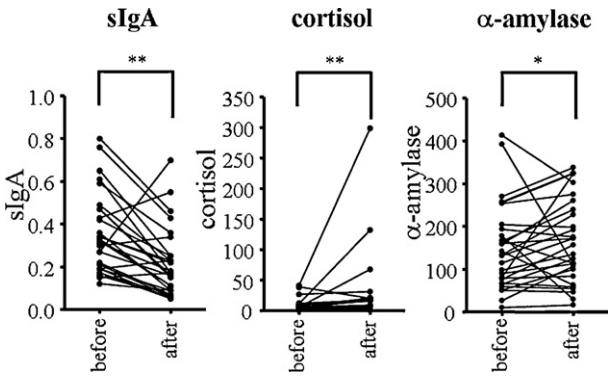

Figure 1 Change in salivary each levels after dental treatment. The values of sIgA, cortisol, and α-amylase measured in samples from 29 subjects are plotted. The *X*-axis represents the sampling time (before: pre-treatment; after: post-treatment). The *Y*-axis represents each levels: the values corrected for the concentration of total protein. $^{*}p < 0.05$, $^{**}p < 0.01$, before treatment vs. after treatment.

to stress. Neuroendocrine regulation plays an important role in the synthesis and secretion of sIgA, such that stimulation of either autonomic (sympathetic and parasympathetic) branch innervating the salivary glands induces a rapid increase (within minutes) in the secretion of sIgA into saliva [33—35]. However, the correlation between the stress and salivary sIgA during dental treatment has not yet been clarified.

The present study is the first to evaluate stress in children caused by dental treatment using the salivary sIgA level [29].

There are some problems with evaluating stress using this measurement system of salivary sIgA. First, it is impossible to measure the salivary sIgA level at the chair-side in the clinic; however, it is possible that detection systems will be developed by another group [36]. Second, the view regarding the correlation between the stress and amount of saliva flow is still controversial. In other words, when sympathetic innervation predominates during stress, the flow rate of saliva decreases [37]. On the contrary, it has been reported that sympathetic activation does not inhibit salivary flow. Third, the view regarding the correlation between the amount of salivary flow and sIgA level is also still controversial. As the distribution of sIgA in each salivary gland is different, the level of sIgA in whole saliva is not correlated with the amount of saliva flow [38]. For the above reasons, it is nonsignificant, even if the amount of whole saliva is measurable.

We have used the concentration of sIgA corrected for the amount of total protein, as the sIgA level is well-correlated with the amount of total protein in saliva [39]. We observed that the overall sIgA level decreased following dental treatment, with significant differences, suggesting that dental treatment represents an undesirable event [29]. Therefore, we are convinced that using salivary sIgA as a stress marker is beneficial for evaluating the change in the sIgA level in each category of the medical records. Although there were no differences between the two groups in the categories not only during pre-treatment but also post-treatment, as assessed by Mann-Whitney *U*-tests, significant differences were detected in the changes after treatment in some categories. Males might be more sensitive, with greater responses in salivary sIgA, compared with females. There is a report that there are no differences in basal salivary sIgA amounts between the sexes; however, the differential responses between the sexes to the stress have not yet been elucidated [40]. It has been reported that salivary sIgA remains relatively stable up to 9 years old [41], or that children do not achieve adults levels of salivary sIgA until they are almost 11 years old [42]; however, the response of salivary sIgA to stress among children has not been elucidated. In this study [29], there was a more significant difference in the younger group (less than 7 years old) than children over 7 years old, suggesting that the fear might decrease with the increasing age of children. There was a significant difference between the treatment times; prolonged treatment ($\geq$20 min) might be more stressful for children. As for the classification of treatment, there was also a significant difference in patients given infiltration anesthesia and the type of surgical operation. As for the children's response to the stressors, there was also a significant difference in children who experienced not only a painful procedure but also a painless procedure, suggesting that a tense feeling while in the chair and the treatment procedure itself are sufficient to induce stress, independent of whether or not the children experienced a painful procedure. However, the results from the use of infiltration anesthesia and painful treatment procedures appear to be inconsistent. The use of infiltration anesthesia might induce a kind of stress in children, whereas painful stimulation might affect the autonomic nervous system and enhance the activity of salivary glands.

Interestingly, salivary sIgA was significantly decreased in children who showed uncooperative behavior and appeared to show few signs of stress; however, those children might be able to effuse some of the stress through their behaviors.

On comparison between sIgA, cortisol, and α-amylase, the sIgA and α-amylase levels showed rapid changes as treatment prolonged or infiltration anesthesia was applied, compared to the cortisol level. In children without maladjustment behavior, the sIgA and cortisol levels were significantly altered by treatment, suggesting that maladjustment behavior reduces stress. However, the α-amylase level did not significantly change.

Conclusion

The salivary sIgA level seems to reflect the degree of latent stress, since it is difficult to comprehend the accurate mental state of children from their descriptions, expressions, and behavior.

In addition, the sIgA and cortisol levels more markedly responded to the stress of dental treatment, compared to the α-amylase level. On a comparison of subjects classified based on their dental records, the sIgA level more sharply responded to stress compared to the cortisol level.

Our results indicate that monitoring the salivary sIgA level is valuable as a noninvasive and sensitive marker of the response to latent stress caused by dental treatment. We are convinced that salivary sIgA is a promising and clinically relevant marker of stress.

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